

Avian Wildlife Reservoir of *Campylobacter fetus* subsp. *jejuni*, *Yersinia* spp., and *Salmonella* spp. in Norway

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Cloacal swabs from 540 wild-living birds were cultured for *Campylobacter fetus* subsp. *jejuni*, *Yersinia* spp., and *Salmonella* spp. The carrier rates detected were as follows: *C. fetus* subsp. *jejuni*, 28.4%; *Yersinia* spp., 1.2%; and *Salmonella* spp., 0.8%. All birds were apparently healthy when captured. *C. fetus* subsp. *jejuni* was isolated from 11 of the 40 bird species examined. Among birds inhabiting the city of Oslo, the highest isolation rate was found in crows (*Corvus corone cornix*) (89.8%), followed by gulls (*Larus* spp.) (50.0%) and domestic pigeons (*Columba livia domesticus*) (4.2%). The gulls and crows scavenge on refuse dumps. High carrier rates were also detected among the following birds from nonurban, coastal areas: puffin (*Fratercula arctica*) (51.3%), common tern (*Sterna hirundo*) (5.6%), common gull (*Larus canus*) (18.9%), black-headed gull (*Larus ridibundus*) (13.2%), and herring gull (*Larus argentatus*) (4.2%). The list of species harboring *C. fetus* subsp. *jejuni* also includes the Ural owl (*Strix uralensis*), goldeneye (*Bucephala clangula*), and reed bunting (*Emberiza schoeniclus*). The following five *Yersinia* strains were isolated: *Y. kristensenii* (two strains), *Y. intermedia* (two strains), and "Yersinia X2" (one strain). Four strains belonging to the genus *Salmonella* were isolated from three different species of gulls. These isolates were identified as *S. typhimurium*, *S. indiana*, and *S. djugu*. The results indicate that campylobacters are a normal component of the intestinal flora in several bird species, whereas *Salmonella* and *Yersinia* carriers are more sporadic.

During the past 20 years, *Yersinia enterocolitica* and *Campylobacter fetus* subsp. *jejuni* have been recognized as important causal agents of human enteric disease (6, 29, 31). There is some evidence that these infections are zoonoses with a world-wide distribution (31). *Y. enterocolitica* and *C. fetus* subsp. *jejuni* have been isolated from a variety of ecological sources, including wild and domestic animals, foods, and the environment (6, 19, 25). However, the epidemiology of these infections is still not completely understood. At present, we do not know the extent to which human infections are derived from animals. The significance of these bacteria in veterinary medicine and in food hygiene requires further evaluation.

Bacteria belonging to the genus *Salmonella* are acknowledged pathogens of considerable medical and economic interest throughout the world (31). The genus comprises about 2,000 serotypes, most of which are zoonotic agents. *Salmonella* spp. are important causal agents of human enteritis and affect a wide range of warm- and cold-blooded animals. Salmonellosis most

commonly results from the ingestion of contaminated food or water.

Due to their great mobility, wild-living birds may function as effective spreaders of disease through fecal contamination of pastures, forage, and surface waters. The present survey was undertaken to assess the avian wildlife reservoir of *C. fetus* subsp. *jejuni*, *Yersinia* spp., and *Salmonella* spp. in Norway.

MATERIALS AND METHODS

Samples. During the period September 1980 to October 1981, cloacal swabs were collected from a total of 540 wild-living birds representing 40 different species (Table 1). The samples were obtained from 25 localities in Norway. The birds studied comprised 138 juveniles and 402 adults (Table 1).

Of the 450 birds examined, 174 originated from the city of Oslo and its suburbs (Table 1). The gulls and crows inhabiting this area scavenge on local refuse dumps. The remaining 366 birds were captured in rural areas, representing a broad spectrum of ecosystems ranging from subarctic and alpine biotopes, through coniferous and deciduous forest areas, to marine habitats.

TABLE 1. Isolation of *C. fetus* subsp. *jejuni*, *Y. enterocolitica*, and *Salmonella* spp. from wild-living birds in Norway (blank space = 0)

Bird ^a	Total no. of samples	No. of samples (%) with		
		<i>Campylobacter</i>	<i>Yersinia</i>	<i>Salmonella</i>
Urban areas				
Domestic pigeon (<i>Columba livia</i>)	71	3 (4.2)	NT ^b	
Raven (<i>Corvus corax</i>)	1		NT	
Hooded crow (<i>Corvus corone cornix</i>)	48	43 (89.8)		NT
Herring gull (<i>Larus argentatus</i>)	19	12 (63.2)	NT	
Black-headed gull (<i>Larus ridibundus</i>)	35	15 (42.9)	NT	2
Rural areas				
Tengmalm's owl (<i>Aegolius funereus</i>) ^c	12		2	
Goldeneye (<i>Bucephala clangula</i>)	1	1		
Hooded crow (<i>C. corone cornix</i>)	1	1		
Reed bunting (<i>Emberiza schoeniclus</i>)	5	1	1	
Puffin (<i>Fratercula arctica</i>)	76	39 (51.3)		
Herring gull (<i>L. argentatus</i>) ^d	24	1 (4.2)		1
Common gull (<i>Larus canus</i>) ^d	37	7 (18.9)		
Lesser black-backed gull (<i>Larus fuscus</i>) ^d	8			
Great black-backed gull (<i>Larus marinus</i>) ^d	4			1
Black-headed gull (<i>Larus ridibundus</i>) ^d	53	7 (13.2)	1	
Common tern (<i>Sterna hirundo</i>)	36	2 (5.6)	1	
Ural owl (<i>Strix uralensis</i>) ^e	2	2		

^a The following 26 species gave negative results (number of individuals in parentheses): meadow pipit (*Anthus pratensis*) (1), scarlet rosefinch (*Carpodacus erythrinus*) (1), house martin (*Delichon urbica*) (9), yellowhammer (*Emberiza citrinella*) (1), pied flycatcher (*Ficedula hypoleuca*) (2), chaffinch (*Fringilla coelebs*) (2), icterine warbler (*Hippolais icterina*) (2), swallow (*Hirundo rustica*) (2), wryneck (*Jynx torquilla*) (3), willow grouse and ptarmigan (*Lagopus* spp.) (37), spotted flycatcher (*Muscicapa striata*) (1), wheatear (*Oenanthe oenanthe*) (1), blue tit (*Parus caeruleus*) (1), great tit (*Parus major*) (1), house sparrow (*Passer domesticus*) (7), tree sparrow (*Passer montanus*) (2), redstart (*Phoenicurus phoenicurus*) (1), willow warbler (*Phylloscopus trochilus*) (3), green woodpecker (*Picus viridis*) (1), whinchat (*Saxicola rubetra*) (2), starling (*Sturnus vulgaris*) (2), hawk owl (*Surnia ulula*) (1), blackcap (*Sylvia atricapilla*) (1), garden warbler (*Sylvia borin*) (12), whitethroat (*Sylvia communis*) (8), fieldfare (*Turdus pilaris*) (3). The birds included in the table are adults unless otherwise stated.

^b NT, Not tested.

^c Ten adults and two juveniles.

^d Juvenile gulls inhabiting the Hvaler Islands.

^e Two juveniles.

Two distinct groups of seagulls (*Larus* spp.) were investigated: (i) 54 adult gulls that scavenge on Grønmo municipal refuse dump in the city of Oslo, where sewage sludge is deposited, and (ii) 125 juvenile gulls that inhabit coastal areas on the Hvaler Islands.

The cloacal swabs were stored in SIFF transport medium (24). Cultivation was performed within 4 days. The samples were examined for the presence of *C. fetus* subsp. *jejuni*, *Yersinia* spp., and *Salmonella* spp.

Isolation of campylobacters. Each sample was plated out onto a selective medium that consisted of the gonococci agar base of Ødegaard (20), defibrinated horse blood (70 ml/liter), IsoVitalX enrichment (BBL Microbiology Systems, Cockeysville, Md.), and the antimicrobial agents colistin (Colimycin, Lundbeck & Co., Copenhagen, Denmark) (10 IU/ml), cefalotin (Keflin, Eli Lilly France S. A., Fegersheim, France) (15 µg/ml), and nystatin (Mycostatin, E. R. Squibb & Sons Ltd., Twickenham, Middlesex, England) (25 IU/ml). Incubation was performed at 42 to 43°C in a microaerobic atmosphere by using the GasPak system (BBL) without a catalyst. The plates were examined after 48 and 72 h. Plates showing no growth were incubated further and read after 1 week.

All colonies morphologically similar to *Campylobacter* spp. were examined by phase-contrast microscopy (×1,000). Bacteria showing the typical motility and cell morphology suggestive of *Campylobacter* spp. were subcultured and subjected to cultural and biochemical examination. The ability to grow under aerobic or anaerobic conditions was assessed after incubation at 42°C for 48 h. Growth at 25°C was tested in a microaerobic atmosphere. Catalase activity was tested on microscopic slides by additions of 1 drop of H₂O₂. Oxidase activity was examined on filter paper with 1% aqueous solution of tetramethyl-*p*-phenylenediamine dihydrochloride. Hydrolysis of hippurate was tested by the rapid method of Hwang and Ederer (9) as modified by Skirrow and Benjamin (26). Sensitivity to nalidixic acid was evaluated on blood agar by means of commercial antibiotic disks (Neo-Sensitabs; A/S Rosco, Taastrup, Denmark) containing 130 µg of nalidixic acid. Bacteria showing inhibition zones of ≥28 mm after incubation at 37°C for 24 h were considered sensitive.

The parameters listed above formed the basis for identification of the isolated strains according to established criteria (28) and enabled allocation to the taxonomic divisions proposed by Skirrow and Benjamin

TABLE 2. Relative prevalence of *C. jejuni*, *C. coli*, and NARTC in wild-living birds^a

Bird	No. of isolates			
	Total	<i>C. jejuni</i>	<i>C. coli</i>	NARTC
Gulls (<i>Larus</i> spp.)	45 ^b	24	12	9
Hooded crow (<i>C. corone cornix</i>)	44	41	2	1
Puffin (<i>F. arctica</i>)	40 ^c		1	39
Pigeon (<i>C. livia</i>)	3	3		
Ural owl (<i>S. uralensis</i>)	2	2		
Common tern (<i>S. hirundo</i>)	2	1	1	
Goldeneye (<i>B. clangula</i>)	1		1	
Reed bunting (<i>E. schoeniclus</i>)	1	1		

^a Isolates were identified by the method of Skirrow and Benjamin (26). Total percent recovery rates were as follows: *C. jejuni*, 52%; *C. coli*, 12.3%; NARTC; 35.5% Blank space = 0.

^b Three birds harbored two biochemically distinct strains (*C. jejuni* and NARTC).

^c One bird harbored two biochemically distinct strains (*C. coli* and NARTC).

(26): *Campylobacter jejuni*, *Campylobacter coli*, and nalidixic acid-resistant thermophilic campylobacters (NARTC).

Isolation of *Yersinia* spp. Cultivation was performed on lactose-bromothymol blue agar (Drigalski agar) and lactose-sucrose-urea agar (10). The agar plates were incubated at room temperature for 48 h. *Y. enterocolitica* and *Y. enterocolitica*-like bacteria were identified on the basis of morphological, cultural, and biochemical characteristics as described previously (11, 12). The isolates were subsequently ascribed to the nomenclature *Y. enterocolitica sensu stricto*, *Y. frederiksenii*, *Y. kristensenii*, or *Y. intermedia*, as defined by Brenner et al. (5). Virulence was evaluated by the autoagglutination test of Laird and Cavanaugh (15).

Isolation of *Salmonella* spp. The cloacal swabs were incubated in selenite broth at 41.5°C. Subcultures were made on brilliant green-lactose-phenol red agar after 48 and 72 h. The agar plates were incubated at 37°C for 24 h. *Salmonella* spp. were identified by means of cultural, biochemical, and serological characteristics by standard procedures (16).

RESULTS

C. fetus subsp. *jejuni* was isolated from 134 (28.4%) of the 540 birds examined (Table 1). Five birds (1.2%) harbored *Y. enterocolitica*-like bacteria. *Salmonella* spp. were recovered from four birds (0.8%). All birds were apparently healthy when captured. Detailed pathological examination was not attempted.

C. fetus subsp. *jejuni*. *C. fetus* subsp. *jejuni* was isolated from 11 of the 40 bird species examined (Table 1). The frequency of isolation from different species is shown in Table 1. The highest isolation rate among the birds inhabiting the city of Oslo was found in crows (*Corvus corone cornix*) (89.8%), followed by gulls (*Larus* spp.) (average, 50.0%), and domestic pigeons (*Columba livia domestica*) (4.2%). These differences were statistically significant ($\chi^2 = 83.76$; $P < 0.0001$). Relatively high rates (51.3 to 4.2%) were also observed among birds originating from nonurban, coastal areas (Table 1).

The 138 *Campylobacter* strains isolated could be assigned to three biochemically distinct taxa;

(26; Table 2). *C. jejuni* was the most commonly isolated, followed by NARTC and *C. coli*. *C. jejuni* predominated among gulls and crows. In contrast, a majority of the strains isolated from puffins (*Fratercula arctica*) were NARTC. Too few strains were isolated from the remaining bird species to justify conclusions as to the distribution of *C. jejuni*, *C. coli*, and NARTC. All except one of the NARTC isolates were obtained from birds associated with marine ecosystems. The distribution of *C. jejuni*, *C. coli*, and NARTC in urban and coastal gulls was not significantly different.

Three gulls carried two biochemically distinct strains (*C. jejuni* and NARTC). Likewise, two biochemically dissimilar strains (*C. coli* and NARTC) were isolated from one of the puffins examined.

***Yersinia* spp.** Five strains belonging to the genus *Yersinia* were isolated (Table 1). Two strains could be ascribed to *Y. kristensenii*, two were *Y. intermedia*, and one strain belonged to *Yersinia* X2 (fermenting rhamnose, not fermenting sucrose or cellobiose) (5). All isolates were negative in Laird's autoagglutination test, indicating a lack of virulence.

***Salmonella* spp.** Four strains belonging to the genus *Salmonella* were isolated from three different species of gulls (Table 1). The isolates were identified as *S. typhimurium* (two strains), *S. indiana* (one strain), and *S. djugu* (one strain). The frequency of *Salmonella* spp. infection was 3.7% among adult gulls which scavenge on Grønmo municipal refuse dump in the city of Oslo (Table 1). The carrier rate among juvenile gulls (1.5%) inhabiting coastal areas on the Hvaler Islands was not significantly lower ($\chi^2 = 0.03$; $P = 0.72$).

DISCUSSION

Y. enterocolitica and *Y. enterocolitica*-like bacteria are frequently encountered in both terrestrial and freshwater ecosystems (12, 19).

However, most strains recovered from animals or environmental sources probably lack clinical significance. The ability to provoke disease has mainly been associated with only a few variants (3, 19). The *Yersinia* spp. strains isolated from birds in this study were *Y. kristensenii*, *Y. intermedia*, and *Yersinia* X2, which differ in biochemical properties from strains considered relevant to human or veterinary medicine (5). These isolates were negative in the autoagglutination test, which is a useful presumptive indication of the pathogenic potential of *Yersinia* spp. isolates (15). Nevertheless, strains similar to those reported in the present study have occasionally been associated with atypical clinical syndromes mainly affecting patients with compromised host defence (3, 4, 19). Furthermore, it has been recently suggested that *Y. kristensenii* may be a possible agent of food intoxication (12a, 14). The clinical significance of *Y. enterocolitica*-like bacteria in human and veterinary medicine may require further evaluation.

The isolation rate of yersiniae from birds (1.2%) was low as compared with the frequencies reported from small rodents (13%) and fish (19%) in Norway (11, 13). On the other hand, *C. fetus* subsp. *jejuni* was commonly found (24.8%) in the birds examined (Table 1). Campylobacters seem to be sparsely represented among small rodents (unpublished results). These observed differences may be attributable to dissimilar temperature preferences. The relative high body temperature of birds may favor the growth of thermophilic campylobacters, whereas *Y. enterocolitica* and *Y. enterocolitica*-like bacteria prevail at lower temperatures, e.g., in rodents, fish, and water (Kapperud, Doctoral thesis, University of Oslo, 1980).

Poultry probably constitutes one of the largest potential reservoirs of *C. fetus* subsp. *jejuni* (6, 23, 29). Among wild-living birds, these bacteria have previously been isolated from pigeons, blackbirds, starlings, and sparrows (27), seagulls and dunlins (25), and some species of migratory waterfowl (17). The present results add several new hosts to those previously listed (Table 1). Hence, our observations further substantiate the suggestion that avian wildlife constitutes an extensive reservoir of *C. fetus* subsp. *jejuni*. The broad spectrum of ecosystems represented might indicate that the occurrence of this bacterium in the environment and in nonavian wildlife may be far greater than currently recognized.

Crows, gulls, and pigeons captured in the city of Oslo showed significantly different carrier rates of campylobacters (Table 1). Dietary variation may account for these differences. The lowest incidence (4.2%) was found in pigeons, which are herbivorous. Crows and gulls, which are omnivorous, showed rates of 90 and 50%,

respectively. An analogous relationship between dietary intake and isolation rate of campylobacters has been detected among migratory waterfowl in Colorado (17).

The populations of gulls and crows examined scavenge on local refuse tips where sewage sludge is deposited. The gulls in question also frequent areas close to drinking water reservoirs, thereby enabling fecal contamination of the water supply and subsequent spread of pathogens to other species, including the human population. Extensive water-borne outbreaks of *Campylobacter* enteritis, affecting several thousand people, have been reported (18, 30). In an epidemic outbreak of human campylobacteriosis in northern Norway, a water reservoir frequented by gulls was considered to be the most probable source of infection (Infectious Disease Control Department, Weekly Report [MSIS], no. 35, 36, 38/1981, NIPH, Oslo). Mæland (A. Mæland, abstr. no. P44, International Workshop on Campylobacter Infections, University of Reading, England, 1981) found that one-third of the cases of human *Campylobacter* enteritis in Oslo originated in the community, whereas two-thirds of the cases were imported. The reservoir was not identified. The present results indicate that wild-living birds may be a potential source of human infection in the Oslo region. The observed biotype profile would seem to support this.

Skirrow and Benjamin (26) recognized four biochemically distinct taxa among *C. fetus* subsp. *jejuni*, which they named *C. jejuni* biotypes 1 and 2, *C. coli*, and NARTC. *C. jejuni*, which constituted 52.2% of the strains isolated in this study (Table 2), is the most frequently encountered variant in human campylobacteriosis (23a, 25). *C. coli* is less commonly involved in human infection and seems to be predominant among swine (21, 22, 23a, 25). Most of the NARTC strains were isolated from marine birds (Table 2). Similar results have been reported from England (25). The NARTC strains are salt tolerant (25). This property may represent an adaption enabling enhanced survival in marine ecosystems. NARTC strains have occasionally been recovered from human clinical specimens, but the clinical significance is dubious (25). It is possible, however, that NARTC has contributed to increased mortality among juvenile puffins in northern Norway (G. Kapperud et al., J. Wild-life Dis., in press).

In accordance with the observations of other authors (25), the birds in this study were apparently healthy. This observation may be biased, however, since the trapping methods tend to select active, healthy individuals. Although *C. fetus* subsp. *jejuni* has been associated with hepatitis and bluecomb enteritis in poultry (29),

the clinical significance for both wild and domestic birds is largely unknown.

Extensive outbreaks of salmonellosis among wild-living birds have been documented in Norway and Sweden (2, 7, 8). All isolates of *Salmonella* spp. in this study were obtained from gulls which were apparently healthy (Table 1). In previous investigations of gulls from coastal areas in Norway, the carrier rate of *Salmonella* spp. was found to be less than 1% (1, 7). Significantly higher rates have been reported in gulls scavenging on refuse tips (1, 7). Although in this study *Campylobacter* was less frequently found among coastal gulls than among populations which fed on a municipal garbage dump, the prevalence of *Salmonella* spp. was not significantly different (Table 1). However, these data are not directly comparable since different age groups were represented. Furthermore, the populations of coastal gulls investigated also have access to local garbage dumps during parts of their life cycle (especially *L. fuscus*, *L. argentatus*, and *L. ridibundus*). Although the population of common gulls (*L. canus*) that was studied never feeds on garbage, sewage effluent represents a significant food supply (O. J. Hansen, personal communication). This population showed the highest incidence of campylobacters (18.9%) among the coastal gulls examined (Table 1).

In conclusion, the present results indicate that campylobacters are a normal component of the enteric flora of several avian species, whereas *Salmonella* spp. and *Yersinia* spp. carriers are more sporadic. Feeding habits related to garbage and sewage increase the risk of infection. However, the carrier rate of campylobacters may be high even in species with very little contact with domestic pollution, e.g., puffins. The potential role of wild-living birds in the epidemiology of human campylobacteriosis will require further studies involving comparison of human and avian strains with respect to antigenic and pathogenic properties.

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